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Research Article

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ICAM-1 overexpression counteracts immune-suppression by tumour cell-derived PGE₂ to restore CTL function

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Keywords

Prostaglandin-E2 (PGE2)

Tumour microenvironment (TME)

Cytotoxic T Lymphocyte (CTL)

Intercellular adhesion molecule-1 (ICAM-1) Cyclooxygenase (COX-2)

ABSTRACT

Tumour-infiltrating cytotoxic T lymphocytes (CTLs) play a key role in tumour killing. However, many cancers adopt various strategies to induce immunosuppression. Priming of naïve CD8⁺ T cells to become CTLs occurs via cognate interactions of the T cell receptor (TcR) and CD28 with tumour-derived peptide epitopes expressed on major histocompatibility complex (MHC) class I molecules and CD80/CD86 on T cells and antigen-presenting cells (APCs) respectively. Here we report that, in the absence of CD80/CD86 expression by renal carcinoma (Renca) cells, expression of intercellular adhesion molecule-1 (ICAM-1) by Renca cells provides a potent alternative co-stimulation to a tumour-specific CD8⁺ T cells causing them to produce interferon gamma (IFN-γ) which is crucial for the further up-regulation of ICAM-1 on tumour cells. We have shown that overexpression of cyclooxygenase-2 (COX-2), by Renca cells (Renca-T3), results in increased levels of prostaglandin (PG) E₂ production, which can directly suppress anti-tumour CD8⁺ T cells resulting in loss of CTL function *in vivo* and cause metastases to the tumor-draining lymph nodes (TDLNs). Significantly, our data also show that overexpression of ICAM-1 on Renca-T3 cells can counteract the immune-suppressive effect of PGE₂ and restore CTL responses.

Introduction

Anti-tumour CD8⁺ cytotoxic T lymphocytes (CTLs) are capable of producing interferon gamma (IFN-γ) and cytolytic enzymes and are crucial for cancer regression¹. Priming naïve CD8⁺ T cells to become CTLs requires two signals; the first comes from T cell receptor (TcR) interaction with peptide epitope presented by major histocompatibility complex (MHC-I) molecules on antigen-presenting cells (APCs). The second signal was long thought to be solely provided by the interaction of T cell-expressed CD28 with the classical co-stimulatory CD80/CD86 molecules on APCs. However, CD80- and CD86-mediated co-stimulation is not provided by most tumour cells. Thus, the direct interaction between naïve CD8⁺ T cells and tumour cells often leads to tolerance induction. In the absence of CD80/CD86², intercellular adhesion molecule-1 (ICAM-1) interaction with lymphocyte function associated antigen-1 (LFA-1), expressed by T cells, can provide a co-stimulatory signal resulting in the formation of CTLs^{3,4}.

ICAM-1 is a member of immunoglobulin superfamily expressed by many cells including various tumour cells⁵. The main role of ICAM-1 is to firmly arrest leukocytes and facilitate extravasation through

the blood vessels to sites of inflammation. However, ICAM-1 also enables T cells to adhere to other cells including APC, endothelial cells, and to normal and tumour cells⁶. In some cancer patients elevated levels of soluble (s) ICAM-1 of around 3 to 5-fold are often found. However, Circulating sICAM-1 is shown to block LFA-1; thus promoting tumour growth and angiogenesis⁷. In contrast, ICAM-1 and/or ICAM-2 negative pancreatic cancer cells are resistant to killing by $\gamma\delta$ T cells. However, such resistance to killing can be reversed by re-expressing ICAM-1 or ICAM-2⁸.

Despite the induction of tumour-specific CTL responses by alternative co-stimulation pathways, tumours still develop various strategies to escape anti-tumour immune responses, such as the over-production of prostaglandin (PG) E_2 as a result of the up-regulation of the cyclooxygenase-2 (COX-2) gene^{9,10}. PGE_2 is a known immune modulator which is able to maintain dendritic cells (DC) in an immature state by: increasing the levels of IL-10 within the tumour microenvironment; enhancing Th17 T cell responses, and recruiting myeloid-derived suppressor cells (MDSC)¹¹. In addition, coupling PGE_2 with cognate receptors on T cells can increase cyclic adenosine-monophosphate (cAMP) within T cells which, consequently, reduces T cell proliferation and effector function¹². Moreover, PGE_2 decreases IFN- γ production by T cells. IFN- γ is shown to be involved in enhancing tumour immunogenicity. PGE_2 can indirectly affect the expression of ICAM-1 by preventing IFN- γ mediated up-regulation of ICAM-1. Therefore, we hypothesized that over-expressing ICAM-1 on COX-2-overexpressing Renca-T3 cells would counteract the immunosuppressive effect of PGE_2 on tumour-specific CD8⁺ T cells.

To test this hypothesis, and to explore the efficacy of ICAM-1 in providing co-stimulatory signals to tumour-specific CD8⁺ T cells, our lab adapted a well-established murine renal carcinoma (Renca) model to generate cells that not only express the haemagglutinin (HA) protein from influenza virus A/PR/8/H1N1 (PR8) as a neo-tumour-specific antigen (Renca-HA;³), but which also over-express COX-2, resulting in the over-production of PGE_2 (Renca-T3;¹²).

Materials and methods

Mice

6 to 8 wk old Thy1.1^{+/+} CL4^{+/-} TcR transgenic BALB/c mice (CL4 mice;¹³) were bred and housed under specific pathogen-free conditions at the University of Bristol Animal Services Unit. All experimental procedures were conducted in accordance with U.K. Home Office guidelines.

Murine renal carcinoma (Renca) tumour cells

Renca-wild type (WT) were cultured in complete media consisting of RPMI (1640) (Sigma- Aldrich, Poole, UK)

supplemented with 10 % (v/v) foetal calf serum (FCS), 2 mM L-glutamine, 50 U/ml penicillin/streptomycin (Life technologies, Paisley, UK), 5×10^{-5} M 2-mercapto-ethanol (Sigma-Aldrich, St. Louis, USA). The Renca-HA cells used throughout this study expressed low levels of HA, (Renca-HA¹⁰; as described in³), and were grown in complete medium supplemented with 100 μ g/ml geneticin (G418; Sigma-Aldrich), and for Renca-T3 medium was additionally supplemented with puromycin (1 μ g/mL; Biomatik). Renca-HA/ICAM-1 and Renca-T3/ICAM-1 cells were generated by transfecting tumour cells with 1.5 μ g of the ICAM-1-expressing vector: pIREShyg (a kind gift from Prof. Adrian Whitehouse at Leeds University) and ICAM-1 cDNA (Sino Biological Inc. Beijing, China) by lipofection. All Renca cells were then left to grow in complete media additionally supplemented with 250 μ g/ml of hygromycin B. Resistant colonies were picked and examined for ICAM-1 expression by flowcytometry. Both HA and ICAM-1 expression were routinely checked during this study.

Enrichment and proliferation of CL4 CD8⁺ T cells

Naïve CL4 CD8⁺ T cells were purified from peripheral lymphoid tissues isolated from CL4 TcR transgenic mice by magnetic-activated cell sorting (MACS) according to the manufacturer's instructions (Miltenyi Biotec Ltd., Bisley, UK). In some instances, naïve CL4 T cells were labeled with 5 μ M carboxyfluorescein succinimidyl ester (CFSE; BioLegend, San Diego, USA). MACS-purified CL4 T cell proliferation was detected either; by a ³H-thymidine-based proliferation assay, whereby 1 μ Ci ³H-thymidine was added in the last 8 hours and the proliferation was measured by thymidine incorporation (counts per minute; cmp) (Amersham Life Science, London, UK), or by a CFSE-based proliferation assay whereby CL4 proliferation was detected by the loss of CFSE expression. Naïve CL4 T cells were activated with; 10 μ g/ml of anti-CD3 mAbs plus either; 5 μ g/ml of anti-CD28 mAbs, or 3 μ g/ml of recombinant (r) ICAM-1, or populations of γ -irradiated Renca cells. Renca cells were irradiated with 9600 RADs using a Cs¹³⁷ source of γ -irradiation (RX30/55; Gravatorm Projects, Grosport, UK).

Flow cytometry

Activated CL4 T cells were either left untreated, or stimulated with 1 μ g/ml K⁴HA peptide and 1 μ g/ml GolgiPlug (BD Bioscience, San Diego, USA), before staining with live/dead aqua and fluorochrome-conjugated monoclonal antibodies (mAbs) for surface staining or they were first permeabilized and then stained intracellularly for IFN- γ .

Statistical analyses

P values were calculated by one-way ANOVA followed by either Dunnett's or Bonferroni's multiple comparison tests using the Prism 5.03 software (GraphPad Software, Inc.).

Results

Characterization of ICAM-1 and LFA-1 expression by naïve CL4 CD8⁺ T cells

To investigate the role of CD8⁺ T cells in anti-tumour immunity, our lab utilized CL4 TcR transgenic mice in which virtually all the CD8⁺ T cells express the V α 10/V β 8.2 TcR transgene¹³. These CL4 T cells are able to recognize the dominant K^d-restricted epitope of the Influenza virus A/PR/8 haemagglutinin (HA) protein (K^dHA_[IYSTVASSL]) expressed by HA-transfected Renca-HA cells³. Naïve CL4 CD8⁺ T cells were purified from the total cells harvested from lymphoid tissues of CL4 mice using the MACS purification technique. This process routinely results in >98% pure CD8⁺ T cells (Figure 1A). ICAM-1 has a central role in naïve CD8⁺ T cell priming³, and acts as a CD28-independent co-stimulator². ICAM-1 interacts with LFA-1 expressed by T cells which is crucial for homotypic T cell aggregation and communication¹⁴. Therefore, MACS-purified naïve CL4 CD8⁺ T cells were stained for ICAM-1 and LFA-1 expression. FACS plots confirm the expression of both ICAM-1 and LFA-1 by naïve CL4 T cells (Figure 1B & C).

Is ICAM-1 a potent alternative co-stimulatory molecule?

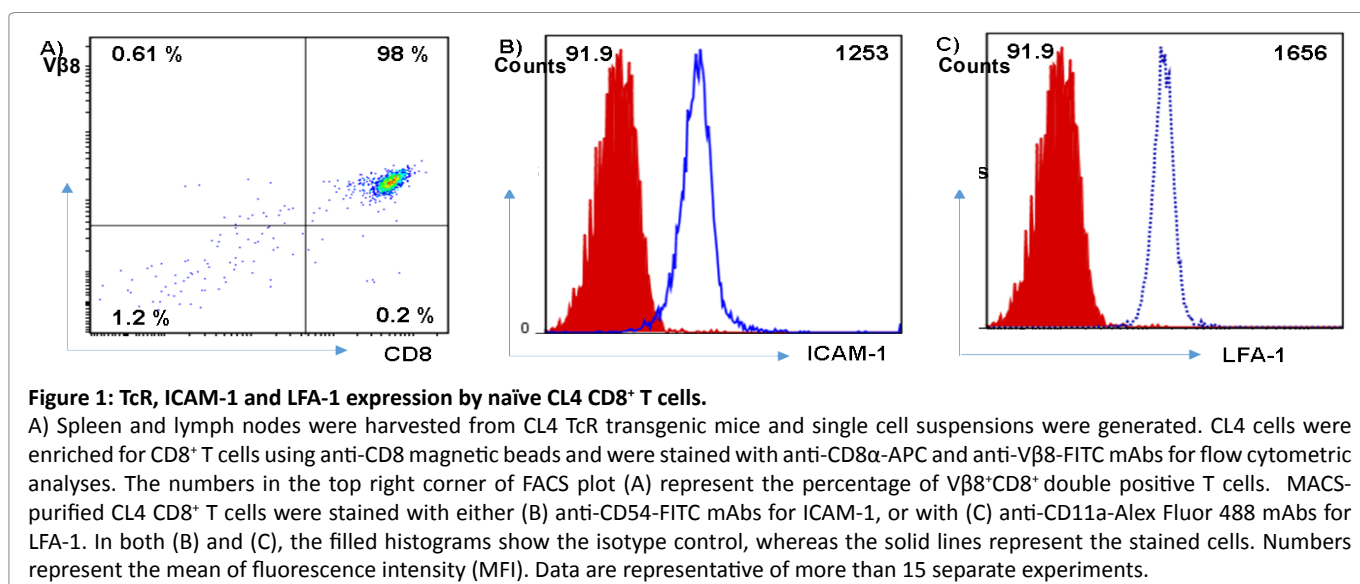
To evaluate the ability of Renca-HA cells, to prime naïve CL4 CD8⁺ T cells, both the HA low-expressing Renca-HA cells³, and control Renca-WT cells were used as APC to prime naïve CL4 T cells in a standard ³H-thymidine incorporation T cell proliferation assay. Whilst naïve CL4 T cells did not proliferate in response to control irradiated Renca-WT APCs *in vitro*, significant levels of CL4 T cell proliferation was observed when cultured with Renca-HA cells as APC (Figure 2A). These data, therefore, suggest that Renca-HA cells are not only able to provide CL4 T cells with signal one but also co-stimulatory signal two. However, we

know that although Renca-HA cells lack CD80/CD86; they do express ICAM-1 (Figure 2B).

To determine if the co-stimulation in our system occurs solely via ICAM-1, anti-ICAM-1 blocking mAbs were added to the CL4 T cells/Renca-HA co-culture. In Figure 3, bar charts show that not only does blocking ICAM-1 significantly decrease CL4 T cell proliferation *in vitro* (A), IFN- γ production is also inhibited; as evidenced by the fact that CL4 T cells were unable to produce IFN- γ compared to the non-treated co-culture (Figure 3B: compare 2.7% with 59%). However, the data show that for both CL4 T cell proliferation in response to classical (anti-CD3 mAbs + anti-CD28 mAbs), and alternative (anti-CD3 mAbs + rICAM-1) priming, only the presence of plate-bound rICAM-1 and not soluble (s) rICAM-1 resulted in enhancement of CL4 T cell proliferation. In addition, treating naïve CL4 T cells with rICAM-1 in solution for an hour before the beginning of the culture is not sufficient to induce the proliferation to a level similar to that achieved using plate-bound rICAM-1 (Figure 3C). Furthermore, there is a positive correlation between the cell surface expression of ICAM-1 on Renca cells and CL4 T cell proliferation as shown in (Figure 3D & E); the more cell surface ICAM-1 that is expressed the more CL4 T cell proliferation is detected. Importantly, these differences in proliferation were not due and changes in the level of HA expression, as after overexpressing ICAM-1, HA expression remained the same for all clones; regardless of the levels of ICAM-1 (Figure 3E).

Can ICAM-1 counteract the inhibitory effect of PGE₂?

Although the basal level of ICAM-1 expression by Renca-HA cells is low, such low level expression is enough to provide sufficient alternative co-stimulation to prime naïve CL4 CD8⁺ T cells *in vitro*. However, despite this, Renca-HA cells continue to grow *in vivo*. Overexpression of COX-2 by Renca-T3 cells resulted in elevated PGE₂



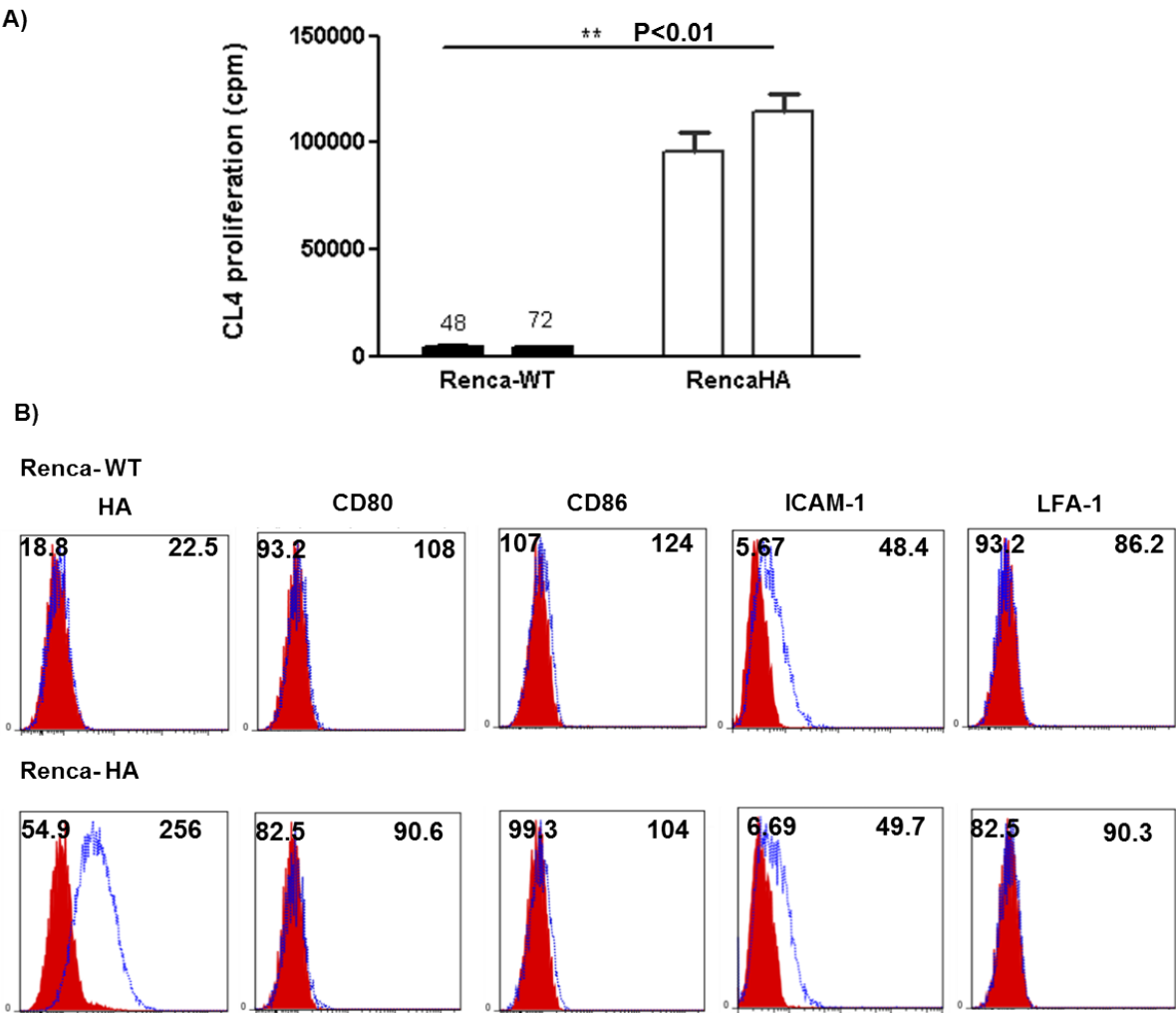


Figure 2: CL4 CD8⁺ T cell proliferation in response to Renca-WT or Renca-HA cells.

A) MACS-purified naïve CL4 CD8⁺ T cells were co-cultured with either irradiated Renca-WT or Renca-HA cells as APCs at a 1:1 effector:target ratio, for 48 and 72 hours. Plates were pulsed with 1 μ Ci of ³H-thymidine in the last 8 hours. After which, CL4 T cells were harvested and the level of ³H-thymidine incorporation was measured. Bar charts show counts per minute (cpm). Error bars represent mean \pm SEM. Data are representative of 3 separate experiments. Statistical analyses were carried out using one-way ANOVA followed by Dunnett's multiple compression test. ** P<0.01

B) Renca-WT and Renca-HA cells were harvested and stained with live/dead aqua and fluorochrome-conjugated mAbs: anti-CD80-FITC, anti-CD86-Alex Fluor 647, anti-CD54-FITC and anti-CD11a -Alex Fluor 488. HA expression was examined using 2-step FACS staining in which Renca cells were incubated first with anti-HA-biotin (Bio), followed by Streptavidin (SA)-PE. Filled lines represent the isotype control, whereas the dotted lines show the indicated surface molecule. Numbers represent the mean of fluorescence intensity (MFI). Data are representative of 3 separate experiments.

production; which has been shown to inhibit anti-tumour responses *in vitro* and *in vivo*^{12,15}. To compare the effect of PGE₂ on the proliferation and the production of IFN- γ by CD8⁺ T cells under classical (anti-CD28), and alternative (rICAM-1), co-stimulation pathways, CFSE-labeled CL4 T cells were primed with either; anti-CD3mAbs + anti-CD28 mAbs, or with anti-CD3 mAbs + plate-bound rICAM-1, in the presence or absence of 1 μ M PGE₂. In the absence of any PGE₂, the FACS plots shown in Figure 4A&B; top row, reveal that around 75% of anti-CD3 mAb-treated CL4 T cells receiving alternative co-stimulation through plate-bound rICAM-1 underwent two or three rounds of division; (each peak showing successive loss of CFSE refers to one round of division). This is compared with only 55% of anti-CD3mAbs-treated CL4 T cells receiving classical costimulatory signaling through anti-CD28 mAbs. This decrease in the proliferation is also associated with a reduction in the number of IFN- γ producing CL4 T cells (Figure 4A&B; bottom row). However, in the presence of PGE₂, there is a decrease in IFN- γ -producing CL4 T cells of nearly 4-fold amongst those cells receiving classical anti-CD28 costimulation; compared to a 2-fold decrease in IFN- γ production among CL4 T cells receiving alternative costimulation via rICAM-1 (Figure 4A&B; bottom row).

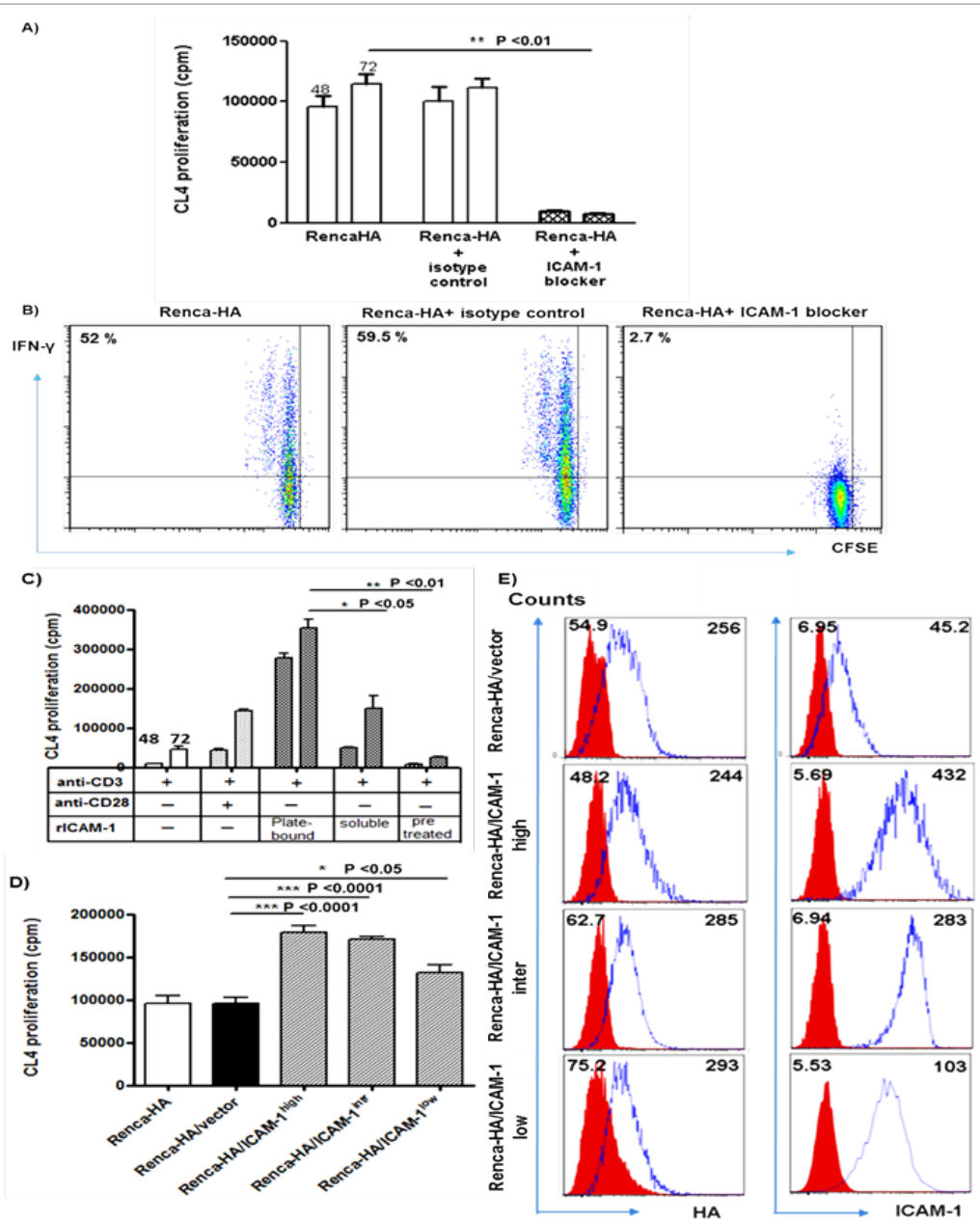


Figure 3: Proliferation and IFN- γ production by CL4 CD8 $^{+}$ T cells in the presence or absence of anti-ICAM-1 blocking mAbs.

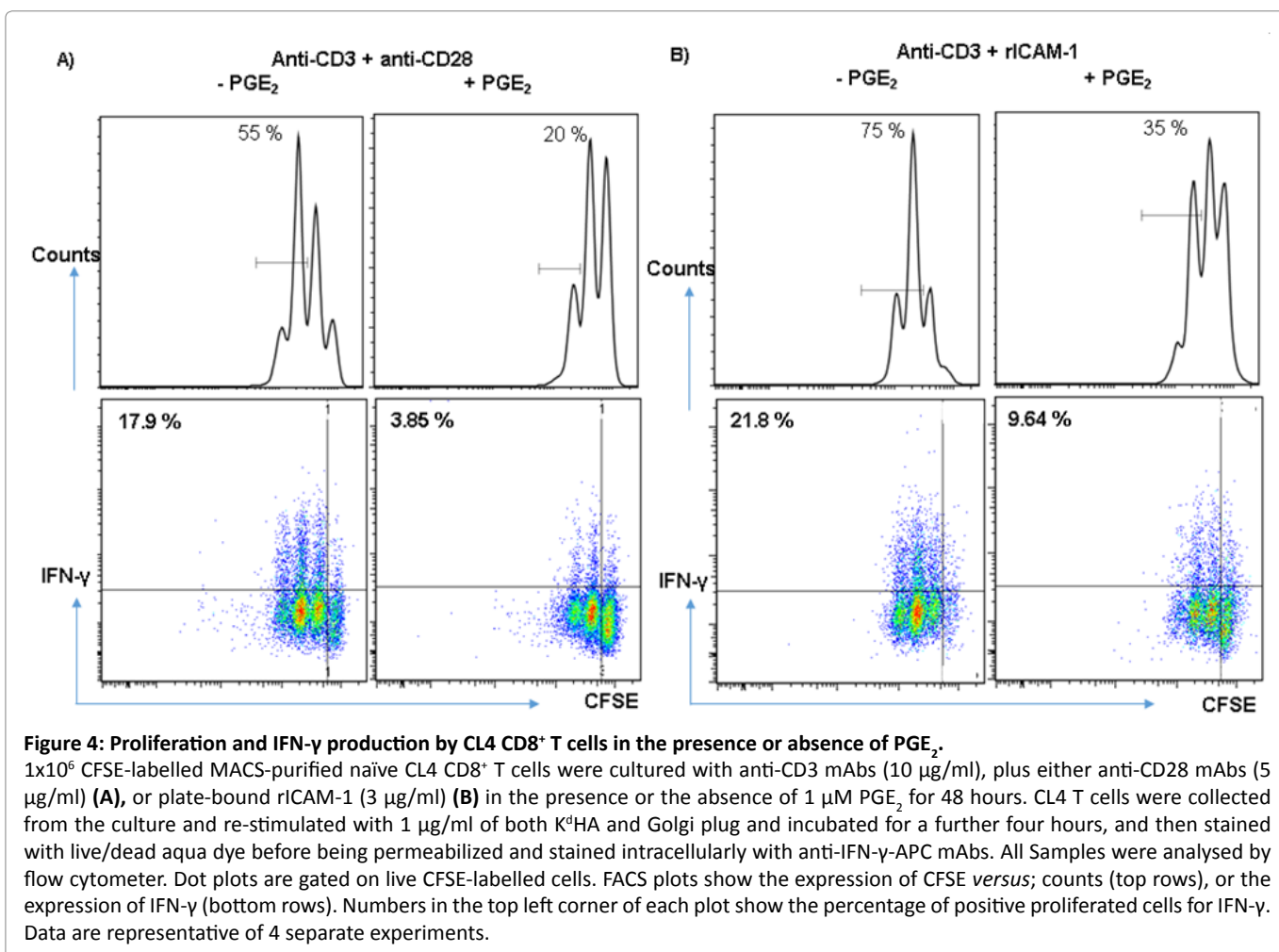
(A) MACS-purified naïve CL4 CD8 $^{+}$ T cells were co-cultured with irradiated Renca-HA at a 1:1 effector: target ratio in the absence or presence of 10 μ g/ml goat anti-mouse ICAM-1/CD54 antibody, or with goat IgG isotype control. During the final 8 hours, cultures were pulsed with 1 μ Ci 3 H-thymidine. CL4 cells were harvested and analysed for 3 H-thymidine incorporation. Bar charts show counts per minute (cpm). Error bars are mean \pm SEM. Data are representative of 3 separate experiments. Statistical analyses were carried out using one way ANOVA followed by Dunnett's multiple comparison test. $** P < 0.01$.

(B) 1×10^6 MACS-purified naïve CL4 CD8 $^{+}$ T cells were co-labelled with CFSE and cultured with 1×10^5 irradiated Renca-HA cells in the absence or presence of 10 μ g/ml goat anti-mouse ICAM-1 blocking mAbs, or with goat IgG isotype control mAbs, for 72 hours. CL4 T cells were then collected re-stimulated with 1 μ g/ml of K c HA and Golgi plug for four hours, and then stained with live/dead aqua before being permeabilized and stained intracellularly with anti-IFN- γ -APC mAbs. FACS plots show the expression of CFSE *versus* the production of IFN- γ by CL4 T cells. The numbers in each FACS plot show the percentage of proliferated CL4 T cells that are also IFN- γ -positive (top left corner). Data are representative of 3 separate experiments.

(C) 1×10^4 MACS-purified naïve CL4 CD8 $^{+}$ T cells were primed with plate-bound anti-CD3 mAbs (10 μ g/ml) alone, or in a combination with either anti-CD28 mAbs (5 μ g/ml) or plate-bound or soluble rICAM-1 (3 μ g/ml). A proportion of CL4 T cells were pre-treated with rICAM-1 for an hour before the unbound ICAM-1 antibodies were washed, for 48 and 72 hours. Plates were pulsed with 1 μ Ci 3 H-thymidine in the last 8 hours. Bar charts show counts per minute (cpm). Error bars are mean \pm SEM. Data are representative of 4 separate experiments. Statistical analyses were carried out using one way ANOVA followed by Bonferroni multiple comparison test. $** P < 0.01$, $* P < 0.05$.

(D) MACS-purified CL4 CD8 $^{+}$ T cells were co-cultured for 48 and 72 hours with irradiated Renca-HA, Renca-HA/vector and various Renca-HA/ICAM-1 cells expressing relatively high, intermediate & low levels of ICAM-1. During the last 8 hours of each culture cells were pulsed with 1 μ Ci of 3 H-thymidine. Bar charts show counts per minute (cpm), error bars show mean \pm SEM, and all data are representative of 2 separate experiments.

(E) Renca-HA/vector and Renca-HA/ICAM-1 cells that express high, intermediate and low levels of ICAM-1 were harvested and stained with live/dead aqua, HA-biotin (Bio) followed by SA-PE mAbs (left column) and anti-CD54-FITC mAbs (right column). Red filled histograms show the isotype control, whereas the blue histograms represent the HA-stained or ICAM-1-stained cells.



It has been shown that some molecules such as matrix metalloproteinase 9 (MMP-9) can cause ICAM-1 to be shed from the surface of tumour cells and is involved in tumour evasion from the immune surveillance. ICAM-1 provides a docking site for pro-MMP-9 which proteolytically cleaves the extracellular domain of ICAM-1 leading to its release from the cell surface¹⁶. To determine whether or not PGE₂ directly affect the priming of naïve CL4 cells by causing ICAM-1 to be shed from the surface of tumour cells, Renca-HA cells were cultured in the presence of 1 μ M PGE₂ for 72 hours then stained for HA and ICAM-1 expression. Data presented in Figure 5A show that Renca-HA cells maintained the same levels of both HA and ICAM-1 following PGE₂ treatment suggesting that PGE₂ does not cause modulation or shedding of these molecules from the cell surface.

IFN- γ is able to exert its anti-tumour effects by directly acting upon the tumour cells themselves¹⁷; as evidenced by the fact that tumour cells lacking IFN- γ R expression grow in mice despite the presence of effective anti-tumour immune responses, which would otherwise kill IFN- γ R-sufficient tumour cells¹⁸. One mechanism through which IFN- γ is thought to reduce the growth of tumour cells *in vivo* is by

enhancing tumour cell immunogenicity¹⁹. Indeed, IFN- γ has been shown to increase antigen presentation to CD8⁺ T cells by increasing MHC class I and ICAM-1 expression by tumour cells³. Similarly, IFN- γ treatment of Renca-HA cells also resulted in a marked increase in ICAM-1 expression (Figure 5B).

To determine whether or not the up-regulation of ICAM-1 is instrumental in abrogating the effect of PGE₂, we generated an ICAM-1 overexpressing Renca-T3 cell line (Renca-T3/ICAM-1), for co-culture with CFSE-labelled naïve CL4 T cells *in vitro*. The data show that co-culture with this cell line resulted in a significant increase in proliferation of CFSE-labelled naïve CL4 T cells, whereby greater than 65% of cells undergo at least three rounds of divisions compared with around 18% of CL4 T cells co-cultured in the presence of either un-transfected Renca-T3 or Renca-T3 empty-vector control cells (Figure 5C; top row). Moreover, co-culture with Renca-T3/ICAM-1 also resulted in a large increase in CTL effector function as evidence by the fact that around 60% of CL4 cells expressed IFN- γ , compared with only 18% of CL4 cells that were co-cultured in the presence of control Renca-T3 or Renca-T3 empty-vector cells (Figure 5C; bottom row). Taken together, these

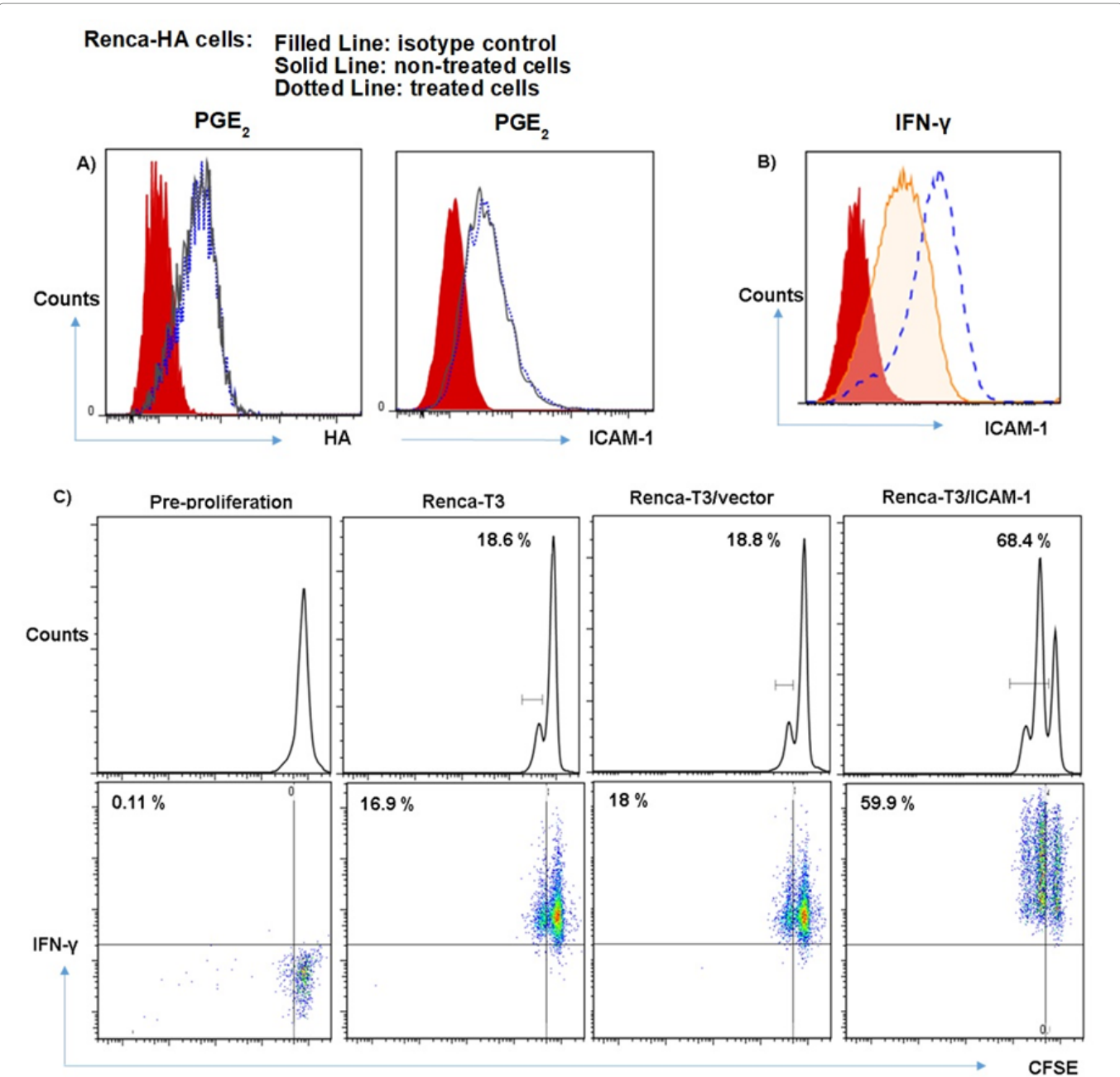


Figure 5: The correlation between IFN-γ, ICAM-1 and PGE₂ in CL4 CD8⁺ T cell response

A) Renca-HA cells were treated with 1 μM exogenous PGE₂ for 72 hours. Renca tumour cells were collected and stained with live/dead aqua, HA-bio followed by SA-PE mAbs and anti-CD54-FITC mAbs. Filled red histograms show the isotype control, whereas the dotted blue lines represent PGE₂-non-treated culture and grey line histograms show PGE₂-treated culture. Data are representative of 4 separate experiments.

B) Renca-HA cells were cultured in the presence or absence of 10 ng/ml IFN-γ for 48 hours. Renca cells were collected and stained with anti-CD54-FITC mAbs and isotype control. Filled lines represent isotype control, whereas solid lines show IFN-γ-non-treated culture, and dashed lines show IFN-γ- treated cells.

C) CFSE-labelled MACS-purified naïve CL4 CD8⁺ T cells were co-cultured with irradiated Renca-T3 and Renca-T3/vector and Renca-T3/ICAM-1 cells for 48 hours. CL4 T cells were collected and re-stimulated with 1 μg/ml of K^aHA and Golgi plug. CL4 T cells were then stained with live/dead aqua before being permeabilized and stained with anti-IFN-γ-APC mAbs. FACS plots show the expression of CFSE *versus* the production of IFN-γ (bottom row), and counts (top row). Numbers in each FACS plot show the percentage of proliferated CL4 T cells that are IFN-γ-positives (top left corner). Data are representative of 3 separate experiments.

data clearly demonstrate that overexpression of ICAM-1 by RencaT3/ICAM-1 cells is able to circumvent the PGE₂-mediated suppression of tumour-specific CTL responses.

Discussion
The requirement of ICAM-1-mediated co-stimulation in T cell priming has remained controversial. Some

reports suggest that ICAM-1 is not sufficient for CD4⁺ T cell activation. In contrast, other reports propose the essential need of ICAM-1 for T cell priming but not for cytokine secretion, whereas others suggest that ICAM-1 can stimulate both activation and cytokine secretion²⁰. However, studies using ICAM-1 knockout mice suggests that ICAM-1 is not essential in T cell-mediated tumour rejection if sufficient numbers of T cells are transferred²¹. In our study, Renca-HA tumour cells, that lack expression of both CD80/CD86, provide K^dHA-specific CL4 CD8⁺ T cells with sufficient co-stimulation through ICAM-1 interactions with T cell-expressed LFA-1 that not only results in proliferation but also in IFN- γ production. This was evidenced by the clear finding that the presence of both anti-ICAM-1 and anti-LFA-1 mAbs during priming significantly reduces CL4 T cell proliferation and IFN- γ production³. Although the interaction of LFA-1 on T cells with soluble or plate-bound rICAM-1 can prime naïve CD8⁺ T cells, only plate-bound rICAM-1 enhances markedly the CL4 T cell response. This finding is consistent with a study in which T cells from mice lacking full-length cell-surface expression of either cell surface ICAM-1 or LFA-1 had significantly impaired T cell function²². It also supports the findings from another study in which over-expression of soluble rICAM-1 blocked LFA-1 on T cells preventing subsequent T cell activation due to the inability of LFA-1 to bind cell-surface ICAM-1. We have shown that overexpressing cell surface ICAM-1 on Renca-HA cells increased CL4 T cell proliferation and IFN- γ . This finding correlates with other studies in which the up-regulation of ICAM-1 on melanoma cells induced by retinoic acid, or ICAM-1 gene transfection, was found to improve the susceptibility of melanoma cells to lysis by lymphokine activated killer cells (LAK)²³. Nevertheless, Renca-HA tumour cells continue to grow *in vivo* when they are injected into BALB/c mice either alone, or with large numbers of naïve CL4 cells. (CN Janicki and DJ Morgan; unpublished data). Following adoptive transfer, CL4 T cells undergo productive activation within the TDLNs, but they lose their effector function once they reach to the tumour site due to the immunosuppression created within the tumour microenvironment²⁴. Many solid tumours create an immunosuppressive microenvironment through various immune escape strategies such as the production of PGE₂. Over-expression of COX-2 not only results in high levels of PGE₂ production, but also tumour cell metastasis by Renca-T3 cells and suppression of activation of naïve CL4 CD8⁺ T cells. Such abortive activation is evidenced by lack of proliferation and the absence of IFN- γ production due to the maintenance of low level ICAM-1 expression¹². Whereas, exposure to IFN- γ was shown to markedly up-regulate ICAM-1. These findings correlate with other studies, which suggest that IFN- γ enhances antigen presentation by tumour cells through the increase of MHC class I expression¹⁸. We showed that disabling PGE₂ production by COX-2 inhibitor has shown to restore

IFN- γ production by effector T cells²⁵. Significantly, over expression of ICAM-1 on Renca-T3 cells restores CL4 T cell proliferation as well as IFN- γ production even in the PGE₂-rich microenvironment. Our findings clearly show that PGE₂ is able to inhibit both the direct priming of naïve tumour-specific CD8⁺ T cells, as well as the effector function amongst a tumour-specific CTL, this effect is temporary and may be mitigated by increasing ICAM-1 expression on tumour cells which therefore enable the ligation of LFA-1 on CL4 with ICAM-1 on tumour cell which then allows the formation of a stable synapse between the two cell types and prolongs this interaction. Along with LFA-1/ICAM-1 binding, the interactions of TcR with K^dHA further increase Ca²⁺ influx and maintain LFA-1 in a high affinity state. In this state, the inhibitory effects, induced by PGE₂-mediated up-regulation of cAMP levels, on Ca²⁺ influx within CL4 T cells fail to override the stimulatory signals through stable LFA-1/ICAM-1 and TcR/K^dHA interactions. As a result, tumour-specific CD8⁺ T cells are forced to proliferate more and produce high levels of IFN- γ and therefore may have greater potential to prevent tumour growth *in vivo*. These findings clearly indicate that drugs which can increase the cell-surface expression of ICAM-1 by tumor cells could provide us with a powerful immune-therapeutic tool to counteract the immunosuppressive action of tumor-derived PGE₂ to greatly enhance anti-tumor CTL responses, which may ultimately control tumor growth.

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